

Joint Research Centre

ERM[®]-AD623: Certified Reference Material for the Quantification of *BCR-ABL1* Transcripts

The JRC in Geel has released the certified reference material (CRM) ERM-AD623 for the calibration of quantitative real time polymerase chain reaction (qPCR) assays measuring the BCR-ABL1 transcript level in cDNA samples from leukaemia patients.

ERM-AD623 is a set of 6 solutions of a double stranded plasmid which is certified to contain a single copy of 3 specific DNA fragments:

- *BCR-ABL1* e14a2 transcript
- *BCR* transcript
- *GUSB* transcript

Each plasmid solution has a certified copy number concentration:

	Copy number concentration of the plasmid	
	Certified value [cp/μL]	Uncertainty [cp/μL]
ERM-AD623a	1.08×10^6	0.13×10^6
ERM-AD623b	1.08×10^5	0.11×10^5
ERM-AD623c	1.03×10^4	0.10×10^4
ERM-AD623d	1.02×10^3	0.09×10^3
ERM-AD623e	1.04×10^2	0.10×10^2
ERM-AD623f	10.0	1.5



Benefits of ERM-AD623 :

- Quantification of *BCR-ABL1* transcripts in relation to 3 control gene transcripts : *ABL1*, *BCR* and *GUSB*
- Suitable for several qPCR assays with different probe/primer sets or qPCR instruments.
- Evaluation of run-to-run variability and trends in results over time
- Improved comparability among results from different laboratories
- Standardised quantification of control gene transcripts for
 - Determination of the limit of detection of the reverse transcriptase qPCR method
 - Assignment of the level of deep molecular response in patients with undetectable *BCR-ABL1* transcript levels
- Assessment of method performance of digital PCR assays

How to order

From JRC in Geel

Tel.: +32 14 571 705 • Fax: +32 14 590 406
<https://ec.europa.eu/jrc/en/reference-materials>
 E-mail: jrc-irrm-rm-distribution@ec.europa.eu

From authorised distributors

LGC Standards GmbH (DE)
<http://www.lgcstandards.com/>
 E-mail: de@lgcstandards.com

Sigma-Aldrich Chemie GmbH (CH)
<http://www.sigmaaldrich.com/irrm>
 E-mail: flukatec@sial.com

Sigma-Aldrich RTC Inc. (USA)
<http://www.RT-Corp.com>
 E-mail: RTCSalesgroup@sial.com

ARMI (USA)
<http://www.armi.com>
 E-mail: Info@ARMi.com

Industrial Analytical (RSA)
<http://www.industrialanalytical.co.za>
 E-mail: info@industrialanalytical.co.za



Accredited CRM Producer: the JRC-IRMM is accredited to ISO Guide 34:2009 for the production of reference materials under the code BELAC 268-RM



<https://ec.europa.eu/jrc/>

Details on ERM-AD623

Material

ERM-AD623 is a set of 6 vials containing solutions of a common plasmid at different copy number concentrations. The plasmid carries three specific DNA fragments, all present as a single copy: one from the fusion transcript *BCR-ABL1* e14a2, one from the native *BCR* transcript and one from the *GUSB* transcript. After linearization by restriction endonuclease digestion, the plasmid was diluted in an aqueous buffer containing 1 mmol/L Tris, 0.01 mmol/L EDTA and 50 ng/μL of transfer RNA from *Escherichia coli*. Each vial contains approximately 600 μL of plasmid solution.

Homogeneity and Stability

The homogeneity, short term and long term stability were demonstrated. Each of the six plasmid solutions was found to be homogeneous both within and between vials provided that the minimal sample intake of 2 μL is respected. The plasmid solutions can be safely stored for two years at -20 °C and transported on dry-ice. Under the condition that contaminations have been excluded, the solutions can be used for several experiments. The material should however not pass more than 10 freeze/thaw cycles. The material can also be stored at 4 °C for up to 4 weeks.

Characterisation

The six plasmid solutions of ERM-AD623 are certified for the number of specific DNA fragments per plasmid and the copy number concentration of the plasmid. The number of specific DNA fragments per plasmid is defined by the sequence of the plasmid as determined by dideoxy terminator sequencing of the entire plasmid. The certified values for the copy number concentration of the plasmid are based on the results from digital PCR measurements performed by three laboratories of demonstrated competence.

Intended use

ERM-AD623 is primarily intended for the calibration of qPCR methods measuring the number of *BCR-ABL1* e14a2 transcripts in relation to the number of transcripts of one of three control genes: *ABL1*, *BCR* or *GUSB*. The six plasmid solutions should be used to construct the calibration curves for both the *BCR-ABL1* transcript and the control gene transcript of choice. The certified copy number concentrations refer to copy numbers of the double stranded plasmid and these values shall be multiplied by 2 when measuring single stranded cDNA samples from patients. In addition, the ERM-AD623 solutions can be used to assess method performance of digital PCR experiments quantifying the number of *BCR-ABL1* transcripts. As any reference material, they can also be used for control charts or validation studies. Detailed instructions for use are given in a dedicated application note (see literature).

Suitability

The suitability of ERM-AD623 as a calibrator for qPCR methods quantifying the level of *BCR-ABL1* e14a2 transcript in cDNA sample has been investigated by several expert laboratories. ERM-AD623 can be used to calibrate several qPCR assays using different probe/primer sets or PCR instruments. If ERM-AD623 is used for the quantification of the *BCR-ABL1* e13a2 transcript, it should be verified that the *BCR-ABL1* e13a2 transcript in the cDNA samples and the *BCR-ABL1* e14a2 transcript in the ERM-AD623 solutions are amplified with equal efficiencies.

Literature:

White H, Deprez L, Corbisier P, et al. 2015, A certified plasmid reference material for the standardisation of *BCR-ABL1* mRNA quantification by real-time quantitative PCR. *Leukemia* 29: 369-376

Liesbet Deprez, Application Note ERM-AD623, European Commission, 2015. available from: <https://ec.europa.eu/jrc/en/reference-materials/application-notes>

Confidence in measurements

All certificates and detailed production information can be found at <https://crm.irmm.jrc.ec.europa.eu>